

**REMARKS/ARGUMENTS**

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The specification has been amended to include the Sequence Listing submitted herewith on separate sheets. The Sequence Listing submitted herewith corresponds to that present in the European application from which the subject application claims priority and the European priority application has been incorporated by reference. The paper and computer readable copies are the same. Entry of the Sequence Listing is not believed to add new matter.

Claims 1-9 have been cancelled without prejudice and new claims 10-27 have been added in lieu thereof. Support for the claims as now presented can be found throughout the application, including in the claims as originally filed (for claim 10, see, for example, paragraph [0019], claims 2 and 5, paragraphs [0009] and [0026] of US 2007/0160711; for claim 14, see, for example, paragraph [0019] of US 2007/0160711; and, for claim 18, see, for example, paragraph [0011] of US 2007/0160711).

Claims 1-9 stand rejected under 35 USC 101. Withdrawal of the rejection is in order in view of the cancellation of claims 1-9. New claims 10-27 are directed to processes and recite positive steps. Reconsideration is requested.

Claims 1-9 stand rejected under 35 USC 112, second paragraph, as allegedly being indefinite. Withdrawal of the rejection is in order for the reasons that follow.

Applicants respectfully submit that the phrase "the flavour development" would be well understood by one skilled in the art. Nonetheless, new claim 10 is drawn to "a

process for providing a more matured taste”, new claim 14 is drawn to “a process for increasing the flavour intensity” and new claim 18 is drawn to “a process for accelerating cheese ripening”.

Furthermore, the claims as now presented do not include narrow ranges falling within broader ranges.

Finally, the claims as presented also do not recite the phrase “for flavor generation”, to which the Examiner objects.

In view of the above, reconsideration is requested.

Claims 1-9 stand rejected under 35 USC 103(a) as allegedly being obvious over Blinkovsky et al (further referred to as R1) and over Transfiguracion et al (further referred to as R2). Withdrawal of both rejections is in order for the reasons that follow.

R1 discloses that *A. oryzae* carboxypeptidase-1 expressed in *F. venenatum* could be a promising candidate for flavor-improving process in the food industry (R1, page 3302 last line). However, it must be kept in mind that R1 deals exclusively with bitterness removal, see, for example, page 3298, left column:

“Commercial use of proteases having different origins for *hydrolysis* of food proteins appears to be very promising .... However, this technology has been hindered by the production of a *bitter* hydrolysate. The *bitterness* has been ascribed to . . . . . It has been shown that *debittering* effects...”

Thus, the sentence on page 3302 must be read in the proper context, that is, the mentioned flavor-improving process refers to a process in which the bitterness is removed. R1 does not teach, nor would it have suggested, anything more. Hence, the carboxypeptidase mentioned in R1 is only associated with debittering.

R1 does not disclose cheese or a process for cheese making, nor that *A. oryzae* carboxypeptidase-1 could be used to increase flavor intensity or accelerate cheese ripening or provide a more mature taste in cheese (see, Example 4, page 9, left column, paragraphs [0018] and [0019] of US 2007/0160711) as compared to a cheese without CPD-1. These results show that CPD-1 accelerates cheese flavor development (see also Example 7, page 5, paragraphs [0023] to [0026] of US 2007/0160711).

R1 describes Flavourzyme as starting material. Flavourzyme is studied in more detail by Kilcawley et al. This study is described in Enzyme Microbial Technology 31:310-320 (2002) and is cited in the subject application. The carboxypeptidase activity of Flavourzyme is zero; (see Kilcawley et al, Table 5). If the skilled person were to use Flavourzyme, he/she would not achieve the effect that the present disclosure teaches, namely, acceleration of cheese ripening as a result of using carboxypeptidase-1 (CPD-1).

R1 does not, therefore, not teach the use of carboxypeptidase-1 (CPD-1) in any of the currently claimed processes.

The Examiner appears to further contend that the skilled person would have added any carboxypeptidase to cheese for flavor improving properties based on the disclosure of R1. However, as indicated below, and as an example, CPD-1 and CPD -Y *do not* have similar properties (for example, they differ in the effect that calcium has on their activity as well as their pH profiles) and can consequently not be interchanged. Therefore, it would not have been obvious to take any carboxypeptidase and apply it in a cheese making process.

Clearly, the claims would not have been obvious over R1.

R2 relates to carboxypeptidase-Y (CPD-Y) from *Kluveromyces fragilis* JSB95 and describes the purification and characterization of CPD-Y from *K. fragilis* JSB95. It is also suggested that CPD-Y might have potential as a useful enzyme for cheese preparation. However, the citation does not provide experimental data in cheese at all.

Applicants submit that the use of CPD-Y from *K. fragilis* JSB95 in cheese preparation would not provide the claimed effect for reasons including the following.

First, it is described in R2 (page 651, second column, lines 6-7 and table 2) that CPD-Y from *K. fragilis* JSB95 is severely inhibited by 1 mM  $\text{Ca}^{2+}$ . During cheese making, calcium is typically added and, as a result, the CPD-Y from *K. fragilis* JSB95 would be inhibited. Surprisingly, the effect of calcium on the activity of carboxypeptidase-1 (CPD-1) is different from the effect of calcium on the activity of carboxypeptidase-Y. Given the data in R2 on activity reduction of CPD-Y in the presence of calcium, the skilled person would not have been encouraged to select carboxypeptidase-Y for flavor development in cheese making, let alone would an artisan have been encouraged to try to use carboxypeptidase-1 instead. The instant application, however, teaches that the CPD-1 activity increases flavor intensity in the presence of calcium (see Example 4 page 9, lines 28-29; Example 5 page 10, line 29; Example 7 page 12, lines 7-8).

Secondly, the pH profile of CPD-Y from *K. fragilis* JSB95 clearly shows that this enzyme has a pH optimum at around pH 6.0 (Figure 4a). The CPD-1 of the present application has a pH optimum around pH 4 and has almost no activity at pH 6.0 (see

Figure 1). The CPD-1 has an activity which is more optimal for the preparation of cheese: the pH during the initial stages of cheese making (approximately the first 2 hours) is around 6.2 to 6.7 where the CPD-1 is essentially inactive and, therefore, can not interfere with the first stage of cheese making. Especially important is that the milk clotting process is not affected. In the flowing stages of the cheese making process, the pH drops to 5.0-5.2, where the CPD-1 is active. As indicated above, the CPD-Y has its highest activity around pH 6, where it could interfere with the initial stages of cheese making, which is undesired. During cheese ripening, where the pH is 5.0-5.2 and the enzyme should exert its action, the CPD-Y is barely active.

In conclusion, CPD-Y from *K. fragilis* JSB95 is not suitable for application in cheese whereas CPD-1, surprisingly, is. Accordingly, the present invention would not have been obvious over R2.

In view of the above, reconsideration and withdrawal of both rejections, based on obviousness are requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

DIJK et al  
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Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By:                     /Mary J. Wilson/

Mary J. Wilson  
Reg. No. 32,955

MJW:tat

901 North Glebe Road, 11th Floor  
Arlington, VA 22203-1808  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100